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SOME EGG-LAYING HABITS OF AMPHITRITE ORNATA VERRILL.

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Several years ago while engaged in working out the development of the unfertilized eggs of *Amphitrite* I had occasion to collect large numbers of these worms. Mead, who worked on the cell-lineage of this annelid, had stated that nothing was known of their breeding season but that ripe sexual products could be had at irregular intervals during June, July and August. Collecting therefore during 1902 and 1903 was entirely at random. Incidentally the opportunity came to observe something about their egg-laying habits. During 1907 and 1908 I have used eggs of the same species for the study of other problems and have had a chance to further verify and extend the observations previously made. I wish here to express my appreciation to the directors of the Marine Biological Laboratory for their kindness and encouragement in helping me to prosecute this work.

The observations mentioned in this paper pertain to two questions in particular. I have always experienced considerable difficulty in obtaining, when wanted, mature sexual products of *Amphitrite*. The first question therefore deals with the time of egg-laying, in the hope that future investigators on this species may be saved some trouble and disappointment. The second question is concerned with the manner of depositing eggs. The eggs and sperm float free in the body cavity and these products are usually in various stages of development. This is true even at the time when worms deposit the mature products in a manner apparently normal. How is it possible to retain the cœlomic corpuscles and the unripe eggs and deposit the ripe sexual products? In a number of instances I have observed the act of depositing eggs or sperm in the laboratory and I studied especially the manner of depositing the eggs.

My results in general are as follows. First, the egg-laying reflex is closely associated with the time of spring tide, the height of the season occurring at the time of new or full moon, or within two days after these dates. The best results were obtained in July, *i. e.*, a larger percentage of mature worms may be collected during this month than during any other. In early summer the period of sexual activity tends to occur a day or so later than the time mentioned, while in late summer the period tends to be earlier by the same amount. In regard to the second question I may say that the ripe and unripe products are not kept separate in laying with absolute exactness. Though among the first few hundred eggs deposited it is hard to find even one that is immature, toward the close of any given period of oviposition the immature eggs form a considerable percentage of the total number. But how does the worm keep these eggs apart in the first part of the period? A full consideration of this question is given later.

In order to understand the discussion of the two questions concerned it will first be necessary to say something of the habitat of this form and the environment under which it lives. These worms live in U-shaped, rather tough, mud tubes that break easily in digging. At one of the openings of the tube, sometimes at both, there is a volcano-shaped mound of sand or earth. The two openings are ordinarily from ten to eighteen inches apart and the depth of the lowest part of the tube is about three fourths the distance between the openings. The worms were collected in six different localities in the vicinity of Woods Hole. In these localities at the time of spring tide extreme high water and extreme low water differ by two to three feet. The tubes are found most abundantly on sandy flats which may vary from fine sand to a rocky character; they are also occasionally formed on sandy mud flats where the tide produces little current. The vertical distribution of the tubes is also comparatively limited. At the time of extreme low water tubes are rarely found beyond a depth of twelve or fifteen inches, and very few are found more than twelve inches above this line.

Probably two thirds of the tubes are within six inches, in a vertical direction, of this low water line at spring tide. The worms are more abundant on flats which are somewhat protected from strong currents. When the tide is running and the water comparatively shallow, it is quite common to see the worms at one of the openings, apparently feeding, the mouth just below the pit of the "volcano," and the numerous tentacles extending out for several inches in radial directions. It was found impossible to observe the deposit of sexual products under natural conditions, and this careful description of their habitat is given in order that we may better interpret the results obtained in the laboratory.

I. Time of Egg-Laying.

Verrill in 1871–2 described the occurrence of an annelid in Vineyard Sound to which he gave the name *Amphitrite ornata* Verrill. Several years later he gave the credit for the original description to Joseph Leidy who in 1855 had described what appears to be the same species under the name of *Terebella ornata* Leidy. No mention is made of egg-laying habits in either of these papers. To Mead belongs the credit of recording the first observations of this kind. He writes : "The limits of the breeding season are unknown. Although about eight hundred worms were collected in lots of twenty or thirty between the first of June and the last of August, only seldom were ripe eggs and ripe spermatozoa obtained. It is useless to cut the worms open, for if the sexual products are mature, they will be discharged, usually at about six o'clock in the evening, more often on the day of capture, sometimes the next day." I found little difficulty in verifying most of these results.

Amphitrite must necessarily be collected in the day time and when the tides are low. They were collected in quantities, from twelve to seventy-five specimens in each lot, and it is estimated that about two thousand adult worms were examined in the course of the four seasons during which their habits were under consideration. When dug they were washed free from the mud tube and placed in a bucket of sea-water to be carried to the laboratory. At times the males and females were placed in separate buckets, but this practice was not often followed, for placing the sexes together did not cause them to discharge sexual products. In the laboratory all worms were carefully washed and isolated in separate dishes of sea-water. Eggs rarely fertilize if removed by cutting open the body wall ; if any considerable number are mature they will later be discharged in an apparently normal manner. The quotation from Mead, concerning the scarcity of ripe eggs and ripe spermatozoa, gives an accurate idea of my own results in that particular. But the time of discharge appears to be not so definite as one might think if guided solely by his description. Table I.

Table I.

Time.	A. M.				P. M.									Total.
	9	10	11	12	1	2	3	4	5	6	7	8	9	
Females........			1	1		2	2	3		3	3	1		16
Males..........							1	3		2	2	1		9
Total			1	1		2	3	6		5	5	2		25

Showing time of day when *Amphitrite* begin to deposit sexual products. In the earlier work no record was kept of the males, hence the small number given in this table.

gives little support to his statement in regard to the time of day at which the worms deposit their eggs. As will be observed twelve of these worms, nearly one half of the entire number, deposited their sexual products between 6:00 and 8:30 P. M. An almost equal number shed their products between 2:00 and 5:00 o'clock, and two specimens were ovipositing between 11:00 A. M. and 1:00 P. M. Injury will sometimes cause *Amphitrite* to throw off their eggs, and of course it is possible that these two worms were injured in some way. However another fact must not be overlooked. It was noticed that nearly all worms deposited products from three to five hours after the low tide at which they were collected. This fact is undoubtedly important and probably explains some of the discrepancies found in the table. In a few instances collections were made between 5:00 and 8:00 A. M. ; more frequently they were brought to the laboratory about noon ; but, owing to local conditions, by far the largest number of worms was collected when the low tide occurred in the afternoon. I believe this tendency to deposit eggs in the afternoon or early in the evening may be accounted for in the following way. When low tide occurs near mid-day or early afternoon, the sand flats are more exposed and reach a higher temperature than under ordinary circumstances. Metabolic changes are undoubtedly more active at these times and for a few hours immediately following. As a consequence, if the worms behave in their tubes as they do in the first few hours in the laboratory, the eggs are laid on a rising tide about the time of slack water. This time would be favorable for fertilization on account of the absence of strong currents. Some five hours later, the young blastula is ready to swim, as shown in a previous paper ('06).

Table II.

	Days Before.							Spring Tide.	Days After.							Total.
	7	6	5	4	3	2	1		1	2	3	4	5	6	7	
Females						2	1	6	2	2	1		1		1	16
Males						1	1	4	2	1	1					10
Females (injured)......								2	1				1	1		5
Males (injured)						1		2	1		1		1		1	7
Total..............	0	0	0	0	0	4	2	14	6	3	3	0	3	1	2	38
Number of times collected......	3	4	1	5	4	5	3	7	4	5	6	6	3	3	6	65

Shows time at which eggs and sperm were deposited in reference to spring tide. Also the number of collections made in reference to the same period.

In all sixty-five experiments are here recorded, scattered pretty evenly throughout the summer from June 24 to August 23. In 1902 I was working on the unfertilized egg and few attempts were made at fertilization; hence the data are rather incomplete for that year. At first glance my data do not appear very significant, but one readily notices a general tendency for the oviposition to occur near the time of spring tides. This is especially true in those experiments where I found mature sexual products in greatest abundance. For example, in 1902 the best success in fertilization occurred on July 7, two days after new moon; in 1903 the best results obtained for the season were at the time of new moon and the day following, July 23 and 24; in 1907 best results happened on July 26, two days after full moon; and in 1908 at new moon, July 28. The full meaning of these facts, however, is best brought out in Table II. Here I have shown the day on which eggs and sperm were deposited in the laboratory in reference to spring tide. In all I have recorded 38 instances where *Amphitrite* have shed eggs or sperm in an apparently normal manner. Twelve of these were known to be injured, and consequently their data are unreliable. But of the twenty-six uninjured worms twenty-three (88 per cent.) shed their products on or within two days of spring tide; twenty-four worms (92 per cent.) shed products within three days of spring tide; only one worm deposited eggs five days, another seven days, after the period named. Of the injured worms nearly sixty per cent. deposited products within two days of this period, while thirty-three per cent. missed the spring tide by more than four days. This suggests the possibility that, of those worms given as uninjured, perhaps the last two were injured internally. Again, the table shows that more worms laid eggs or sperm on the day of spring tide than on any other day; that the next largest number deposited products on the day following; that practically all sexual products are deposited within three days of spring tide with a tendency to follow rather than to precede this period. That the distribution of these figures is not due to a like distribution of the number of times worms were collected, is shown clearly by comparing them with the figures in the last line of the table.

In order to make a further test of the hypothesis that ripe sexual products are deposited most abundantly at the time of spring tide, on July 17, 1909, the date of the new moon, twenty adult *Amphitrite* were collected. Of this number twenty-five per cent., three females and two males, deposited ripe products, thus giving excellent confirmation to the hypothesis. The question arises how may we account for the periodicity in the time of egg-laying. Observations that have been made upon some other forms will help in our explanation.

Mayer ('02) has described the interesting case of the breeding habits of the Atlantic palolo. This worm "swarms at the surface before sunrise within three days of the day of the last quarter of the moon between June 29 and July 28." "All eggs mature simultaneously at the time of the normal swarm." Later observations ('09) show that the time of swarming is not so definite as at first supposed. "When the last quarter of the moon falls late in July there may be a response to the first quarter as well as to the last quarter." "A dense swarm occurred on July 10, 1908, a fairly dense swarm on July 19." This behavior shows that a particular change of the moon has no direct effect on the time of swarming, as the moon was in first quarter July 6 and in last quarter July 20. Mayer also performed an important experiment to test the effect of tides on the time of swarming. He writes as follows: "Some worms swarmed normally on July 19 out of the rocks which had been maintained in a floating (tideless) live car for the six weeks previous to the swarm." This experiment appears to demonstrate that tides are without direct effect in producing the swarm. But, as Mayer concludes, it "may indicate that the changing pressure due to rise and fall of tide over the reefs is a contributory but *not a necessary* component of the stimulus which calls forth the breeding swarm."

However, it is known that the tide may form a sort of habit in the action of some animals. Gamble and Keeble ('06) have described a small, green, sedentary turbellarian, *Convoluta roscoffensis*, that occurs on the coast of Brittany. "It exhibits a periodic vertical movement whose rhythm is that of the tide." When the tide is out, they come to the surface, forming green patches on the sand. When the tide comes in they retreat below the surface into safer quarters. The remarkable fact is that when removed from the effects of tidal action by being kept in an aquarium, *Convoluta* continues to perform rhythmic movements in time with the tide. "The rhythm is not profoundly impressed upon it; after a day the movements of the patch in the vessel cease to synchronize with those in the open." This illustration suffices to show how an action may arise in relation to the tide without depending directly upon it.

In *Amphitrite* it has been observed that feeding is more continuous and more active as the time of spring tide approaches. At such times the great mass of tentacles radiate from one opening of the tube and actively explore the vicinity for a distance of several inches. Their food consists of small bits of organic matter carried to the mouth through the action of cilia. One result of the feeding activity is seen in the fact that the immature eggs or sperm grow and mature very rapidly during the last few days immediately preceding the sexual period. Undoubtedly, this increased activity of the organism is stimulated by the higher temperature of the sand flats at the time of spring tide. The alternate increase and decrease in the depth of the water, thus altering the pressure, probably has some influence and the food supply at this period is certainly more abundant. We must conclude therefore that *the influence of the tides or moon is entirely secondary. The sexual activity in these worms is closely associated with a similar rhythmical period of greater bodily activity; and this greater bodily vigor of the animal is induced by conditions that depend upon the tides.* Furthermore, the periodic sexual reflex has acquired a sort of physiological basis in the organism, for the worms deposit sexual products normally, when removed from the influence of the tide. Still this reflex has not become a habit in the animal, at least not a strong one, for if a worm does not deposit its products within a few hours after being captured it rarely does so, and then not later than the following day.

II. Method of Egg-Laying.

The eggs of *Amphitrite* break loose from the matrix of the germinal epithelium in early stages of development and complete their growth while floating free in the cœlomic fluid. In a single worm they are usually found in the various stages of development. When first collected, all worms go through with a series of rhythmic movements of the body. When performed in the tube, these movements are evidently for the purpose of aeration, and they are kept up for some hours after the animals are removed from the tube, gradually diminishing in intensity. Each series of movements begins as a contraction near the posterior end of the body and travels forward; a second contraction follows, and frequently a third has begun before the first has disappeared. Between the contractions are wave-like enlargements that serve as moving valves to pump the water slowly through the tube. This pumping action may be demonstrated by placing a recently captured, uninjured worm in a U-shaped glass tube of suitable size. When *Amphitrite* is about to deposit its eggs the movements become more rapid and frequently more violent than usual. Quoting from my notes, the further process is somewhat as follows: "While the worm's body is undergoing the series of slow peristaltic movements, consisting of contraction-waves that begin near the posterior end and travel forward, the eggs ooze out in string-like, sticky masses that are soon scattered by the movements of the body, or by currents of water. The eggs are extruded through five openings in the anterior region of the body, the first opening being on the second segment back of the third pair of gills, or the sixth body segment, not counting the prestomium. Sperm is extruded through similar openings of the same number and location." These openings are nephridiopores that have become specialized as gonaducts, and it should be added that they are laterally placed, lying between the dorsal and ventral chætigerous lobes.

In a previous paper I have mentioned the fact that the eggs are greatly flattened in the polar diameter at the time of extrusion, and that the first polar spindle is in the metaphase. I have also mentioned above the fact that these worms possess the power of separating ripe and unripe eggs in the process of oviposition. How is this accomplished? Some dissections were made in an attempt to answer the question. It was found that this species possesses the same general arrangement of internal organs as that of other *Amphitrite* described by Meyer ('87). "Alle Nephridien der Terebelloiden, sowohl die vorderen als die hinteren, münden im Bereiche desjenigen Korperzonites, welchem sie angehören, einzeln und unabhangig von einander nach aussen; ihre Wimpertrichter haben eine intersegmental Lage und öffen sich stets in das nächst vorangehende Segment." All the septa in the Terebellidæ are incomplete with one exception; this one, the diaphragm, is strongly developed and separates the anterior region of the body cavity from the rest. In the species here described the diaphragm is between the fourth and fifth body segments. The external openings of the excretory nephridia open on the third, fourth and fifth segments, but the inner openings are all anterior to the diaphragm. The inner openings of the post-diaphragmatic nephridia are fimbriated membranes, consisting of dorsal and ventral portions in close apposition, covered by cilia ; each of these openings leads into a large membranous sac that is well supplied with blood capillaries and is also ciliated. These nephridia serve as gonaducts, and probably serve also as excretory organs. When egg-laying was first observed, I thought the collapse of the egg in the polar diameter might have a direct relation to the separating process, but there is nothing in the structure of the nephridia to indicate that the eggs undergo a sifting process. Besides the cœlomic corpuscles do not escape and they are smaller than either ripe or unripe eggs.

Somewhat similar phenomena have been observed by Gerould ('06) in *Phascolosoma*. "A few hours before egg-laying occurs, the nephridia become distended with a transparent fluid." "Ova that are ready for maturation, having the spindle of the first polar body in metaphase, are swept from the cœlom into the nephridia by the action of cilia which give rise to strong currents within the nephridium, setting from the nephrostome backward towards its posterior extremity. This is a most interesting process in that both the immature ovocytes, which are present in great numbers in the cœlomic fluid, and the cœlomic corpuscles are excluded from the nephridium, while the fully grown ovocytes are collected there in great numbers." Gerould presents a tentative explanation somewhat as follows. The transparent fluid, he thinks, is sea-water, taken in through the nephridiopore, and he believes that ova in the early stages of maturation probably absorb water while within the nephridium. "If eggs in the earliest stages of maturation show a tendency within the nephridium to absorb sea-water, may it not be assumed that ova at that stage are positively hydrotropic ? On this supposition we may explain why such eggs are caught up from the cœlomic currents into the nephrostomal region, and thence carried into the nephridium." This assumption, however, is not to be seriously regarded and is, I believe, incorrect ; at least it is incorrect in the case of *Amphitrite*.

It is rather an easy matter to separate the ripe eggs from the immature ones and from the cœlomic corpuscles by decanting after stirring the mixture in sea-water ; the largest ova and the ripe eggs, those with the first maturation spindle in metaphase, always settle more quickly to the bottom of the dish. The immature eggs then settle, and last of all the cœlomic corpuscles. *It is not an assumption, therefore, to say that the largest ova including those in the early stages of maturation, are more quickly influenced by gravity than the other bodies floating in the cœlomic fluid.* This is the important fact, and is no doubt due to the larger amount of yolk in such eggs.[1]

If, as Gerould supposes, a hydrotropic attraction is necessary to separate these eggs from the other bodies in the cœlomic fluid, then this influence would be useless for the purpose of separation when the contents of the body cavity are emptied in sea-water. For, surrounded by water, the hydrotropic influence would act in all directions and result in equilibrium. Such is not the case. Indeed, Gerould noticed such facts and in appendix B describes how large individuals should be opened in sea-water. "When the female with an abundance of eggs is found, the maturer ovocytes should be allowed to settle to the bottom, whereas the smaller ovocytes and cœlomic corpuscles should be decanted after a few seconds, and before they have had time to sink."

That gravity forms the differential by which the separation takes place is supported by a considerable number of facts. In the course of one period of oviposition, usually extending from one to one and one half hours, the eggs at first deposited are practically all in the metaphase of the first polar spindle ; in the latter part of the period there is always a considerable number, and sometimes a majority, of ova deposited with the germinal vesicle intact. Upon killing a worm that is through egg-laying, one may still find a few scattered eggs in early maturation. If the separation depended upon a tropism, one does not see why it should be so much more complete at one time than another.

[1] Whether this tendency of large ova to settle quickly is due to a greater specific gravity, or to a greater mass in proportion to the amount of surface offering resistance, the end result is the same. This question must be decided by further investigation. In this paper we shall speak of the large ova as though they had a greater specific gravity than the smaller cœlomic bodies.

Again, the nephridia are not extraordinarily large, and each must be refilled many times in the course of one oviposition where thousands of eggs are deposited. Nor do the nephridia ever seem to be entirely filled with eggs; at least such is the case in worms that are killed almost instantly with hot sublimate acetic while in the act of oviposition. The meaning of this will appear in the explanation. It is also manifestly clear that the movements of the body referred to before, cause the body contents to move forward toward the diaphragm. As a rule, each effective contraction-wave stops a short distance posterior to the nephridia and holds for a moment the compressed cœlomic contents in the much distended anterior portion of the body. During the final part of a contraction-wave, a stream of eggs oozes out a short distance from each nephridiopore posterior to the diaphragm. The contraction movements, therefore, are necessary for the expulsion of the eggs.

The explanation which I believe accords best with all the known facts is as follows: First, it should be remembered that the nephridial sacs always occupy a lower position with reference to gravity than the nephrostome, whether the worm lies in a horizontal or vertical direction. We may then think of the nephridia as settling basins in which the heavier products are drawn off from the bottom after a certain amount has accumulated. Ciliary action undoubtedly would prevent lighter objects, such as the cœlomic corpuscles and the unripe eggs from settling in the basin; and when a sufficient quantity of the ripe eggs (heavier objects) have accumulated, pressure from the strong contraction-wave forces them through the nephridiopore. The separating process probably takes place during relaxation, between contraction waves. Owing to the opacity of the worm's body, it is impossible to actually observe this process, but all of the facts point to this simple explanation.

Summary.

1. In *Amphritite ornata* Verrill, the egg-laying reflex is closely associated with the time of spring tide; the height of any given period of egg-laying always occurs within two days of the time of new or full moon. Periods of oviposition occur in June, July and August.

2. The moon does not have any direct influence in producing the period of sexual activity. It is probable that the tide also has little, if any, direct effect on the process.

3. At spring tide, the worms feed more actively, the food supply is more abundant and the sand flats have a higher temperature. As this period approaches we also find a more rapid growth and development of immature eggs and sperm. Therefore, the period of sexual activity is closely associated with a synchronous period of greater bodily activity, and this greater vigor of the animal is induced by conditions that depend upon the tide. In this way we may explain how oviposition in *Amphritite* has become a sort of reflex habit associated with the time of spring tide.

4. When a worm is sexually mature, the cœlomic fluid contains cœlomic corpuscles and eggs in various stages of development. At oviposition the worm extrudes ripe eggs, and toward the end of the process some of the immature ones, but always retains the much smaller cœlomic corpuscles.

5. Since the mature eggs sink faster in sea-water than the smaller immature ones, and all eggs sink faster than the cœlomic corpuscles, it is believed that the larger eggs have a greater density than the other bodies in the cœlomic fluid; and it is entirely probable that the apparent selection of ripe eggs and the rejection of immature ones is due to the different effects produced by nephridial currents upon bodies of apparently different densities.

6. The position of the nephridial sacs, and the arrangement of cilia on the nephrostomes and within the sacs, is such that we may regard the nephridia as a set of settling basins in which the separation takes place. Contractions of the worm's body then aid in expelling the ripe eggs from the nephridial sacs.

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